

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1.-30. (Cancelled)

31. (New) A cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin;
- (iii) fibronectin,
- (iv) tenascin; and,
- (v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components during the culturing conditions; and,

(b) a second layer of keratinocyte cells disposed on the first layer to form an epidermal cell layer, wherein the epidermal cell layer is multilayered, stratified, differentiated and exhibits a basal layer, suprabasal layer, a granular layer and a stratum corneum;

and wherein the bilayered cultured skin construct has a basement membrane present at the junction of the first and second layers.

32. (New) The cultured skin construct of claim 31, wherein said cultured cells are cultured in chemically defined media.

33. (New) The cultured skin construct of claim 31, wherein said first layer has cultured cells from dermal papilla of hair follicles localized on said first layer.

34. (New) The cultured skin construct of claim 31, wherein the cultured dermal fibroblast cells are genetically modified to produce extracellular matrix components.

35. (New) The cultured skin construct of claim 31, wherein the cultured dermal fibroblast cells are genetically modified to produce a growth factor, hormone, peptide, or protein.

36. (New) The cultured skin construct of any of claims 31-35, wherein the construct is cohesive in having physical unitary integrity and tissue-like handling properties.

37. (New) A cultured skin construct having at least two layers, comprising:
(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin;
- (iii) fibronectin,
- (iv) tenascin; and,
- (v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components during the culturing conditions; and,

(b) a second layer of cells comprising epithelial cells disposed on the first layer.

38. (New) The cultured skin construct of claim 37, wherein the epithelial cells are selected from the group consisting of keratinocytes, corneal epithelial

cells, epithelial cells from oral mucosa, esophageal epithelial cells, and uroepithelial cells.

39. (New) A cultured tissue construct having at least three layers, comprising:

(a) a first layer of cultured fibroblasts cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin; and,
- (iii) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components during the culturing conditions;

(b) a second layer of cells comprising epithelial cells disposed on the first layer; and,

(c) a third layer of cells disposed on the second layer of epithelial cells.

40. (New) The cultured tissue construct of claim 39, wherein said fibroblast cells contained within said first layer are derived from tissue selected from the group consisting of neonate male foreskin, dermis, tendon, lung, cartilage, urethra, corneal stroma, oral mucosa, umbilical cord, and intestine.

41. (New) The cultured tissue construct of claim 39, wherein said fibroblast cells contained within said first layer are dermal fibroblasts.

42. (New) The cultured tissue construct of claim 39, wherein said first layer has cultured cells from dermal papilla of hair follicles localized on said first layer.

43. (New) The cultured tissue construct of claim 39, wherein said cultured cells are cultured in chemically defined media.

44. (New) The cultured tissue construct of claim 39, wherein the cultured fibroblast cells are genetically modified to produce extracellular matrix components.

45. (New) The cultured tissue construct of claim 39, wherein the cultured fibroblast cells are genetically modified to produce a growth factor, hormone, peptide, or protein.

46. (New) The cultured tissue construct of claim 39, wherein the epithelial cells are selected from the group consisting of keratinocytes, corneal epithelial cells, epithelial cells from oral mucosa, esophageal epithelial cells, and uroepithelial cells.

47. (New) The cultured tissue construct of any of claims 39-46, wherein the construct is cohesive in having physical unitary integrity and tissue-like handling properties.

48. (New) A method for producing a cultured tissue construct, comprising,

(a) seeding fibroblast cells capable of synthesizing an extracellular matrix on a porous membrane in a culture vessel in a cell culture medium at about 80% to about 100% confluence;

(b) stimulating the fibroblast cells to synthesize, secrete and organize extracellular matrix components in a second culture medium; and,

(c) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of at least about 30 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) tenascin; and,

(iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components during the culturing conditions.

49. (New) The method of claim 48, wherein the fibroblast cells are derived from tissue selected from the group consisting of neonate male foreskin, dermis, tendon, lung, cartilage, urethra, corneal stroma, oral mucosa, umbilical cord, and intestine.

50. (New) A method for transplantation or implantation of a cultured skin construct into a patient comprising transplanting or implanting into a patient, a cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin;

(iii) fibronectin,

(iv) tenascin; and,

(v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components during the culturing conditions; and,

(b) a second layer of keratinocyte cells disposed on the first layer to form an epidermal cell layer, wherein the epidermal cell layer is multilayered, stratified,

differentiated and exhibits a basal layer, suprabasal layer, a granular layer and a stratum corneum;

and wherein the bilayered cultured skin construct has a basement membrane present at the junction of the first and second layers.

51. (New) A method for transplantation or implantation of a cultured skin construct into a patient comprising transplanting or implanting into a patient, a cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin;
- (iii) fibronectin,
- (iv) tenascin; and,
- (v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components during the culturing conditions; and,

(b) a second layer of cells comprising epithelial cells disposed on the first layer.

52. (New) A method for transplantation or implantation of a cultured tissue construct into a patient comprising transplanting or implanting into a patient, a cultured tissue construct having at least three layers, comprising:

(a) a first layer of cultured fibroblasts cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells,

with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin; and,
- (iii) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components during the culturing conditions;

(b) a second layer of cells comprising epithelial cells disposed on the first layer; and,

(c) a third layer of cells disposed on the second layer of epithelial cells.